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# A SYSTEMATIC STUDY OF THE COCCACEAE IN THE COLLECTION OF THE MUSEUM OF NATURAL HISTORY.\*

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Five years ago the Winslows published a book<sup>1</sup> in which they proposed the adoption of the statistical method for the systematic classification of bacteria. They made a careful study of 500 strains of cocci which, together with the similar work on streptococci by Andrewes and Horder,<sup>2</sup> covered the entire family of the Coccaceae. It appeared that by applying a number of morphological and biochemical tests, recording the results quantitatively, wherever possible, and plotting them, the cocci grouped themselves about a few well-defined type centers. The correlation between different characters indicated that there are two main series or subfamilies among the Coccaceae, one primarily of parasitic origin and the other commonly found in water and earth outside the body. Within these two subfamilies, subgroups appeared conveniently characterized by the chromogenesis of their growth on agar. The various physical and physiological properties of the organisms correlated so well with that of pigment production that genera could be broadly distinguished in accordance with this character; and a natural biological grouping was apparently obtained.

Since the appearance of this book various workers have applied the same principle to other groups of bacteria. Winslow,<sup>3</sup> Broadhurst,<sup>4</sup> Stowell and Hilliard<sup>5</sup> have attacked the difficult problem of discovering some order among the streptococci with promise of success. Howe<sup>6</sup> has applied this method in a study of the colon group. Morse<sup>7</sup> has recently published the results of a very successful application of the principles of biometry to the study of the

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<sup>1</sup> *Systematic Relationship of the Coccaceae*, 1908.

<sup>2</sup> *Lancet*, 1906, 2, p. 708.

<sup>3</sup> *Jour. Infect. Dis.*, 1912, 10, p. 285.

<sup>4</sup> *Ibid.*, 1912, 10, p. 272.

<sup>5</sup> *Science* 1912, 35, p. 223.

<sup>6</sup> *Ibid.*, 1912, 35, p. 225.

<sup>7</sup> *Jour. Infect. Dis.*, 1912, 11, p. 253.

diphtheria group. No one has, however, thought it desirable to test, verify, and possibly extend, the results obtained by the Winslows, in regard to the group of the cocci as a whole.

The large number of organisms in our bacterial museum and the facilities thus afforded for systematic work, led me, at the suggestion of Professor Winslow, to undertake a biometric study of the cocci in our collection. The cocci, other than those belonging to the streptococcus and diplococcus groups, numbered 54. These strains were sent to us under 30 different names, representing, according to the classification of Migula and Chester, as many distinct species. It was interesting to see, therefore, whether the biometric classification could be applied to this large assortment of species, and how these supposedly distinct types would group themselves. The results speak for themselves.

The 54 strains in our collection representing the various groups of cocci were put through a series of 10 of the tests found by the Winslows to be of classificatory value. In addition to these, the acid production in a 1 per cent solution of saccharose broth and the ammonification of a 1 per cent solution of peptone were observed. The value of the former test could not be discerned in the small number of organisms tested (in general it behaves similarly to the other disaccharid, lactose), while the latter proved to be highly important.

The methods used were, with slight modifications, those followed by the Winslows. As a detailed discussion is very clearly presented by them, a concise statement of the tests with some brief comments, where necessary, will here suffice.

1. *Habitat*.—Unfortunately only 20 odd strains have a definite history, as to the source of isolation. Where the origin is known, the white and orange forms are generally from the human or animal body, while the yellow and red forms are from air, water, or other saprophytic sources.

2. *Grouping and dimensions*.—Observed on stained specimens from agar streaks after 3 or 4 days at 20° C. and 37° C. respectively. Recorded as: (1) packets, (2) no packets. The results were checked on another occasion from young agar streaks at 20° C.

3. *Gram reaction*.—Observed on three-days-old agar streaks at 20° C. and 37° C. on two different occasions.

4. *Vigor of surface growth*.—After 14 days on agar at 20°. Recorded as (a) faint; (b) meager; (c) good; (d) abundant; (e) very abundant.

5, 6, 7. *Acid produced*, after 14 days at 20° in 1 per cent solutions of dextrose, lactose, and saccharose broth. Determined by titration with N/20 NaOH, using phenolphthalein as indicator and titrating and subtracting value of controls. Results recorded in percentage normal.

8. *Nitrite and ammonia* production in nitrate broth, tested in the usual way, after incubating for 14 days at 20° C. Ten tubes were used for each strain. About 25 of the cultures were repeated, incubating for only seven days. No appreciable difference was found, except that in a few instances the intensity of the reaction (hence the amount of the ammonia or nitrite) was decreased. In some cases, notably among the micrococci, ammonia only was produced, and repeated tests failed to show the presence of nitrites. Winslow<sup>1</sup> obtained similar results and was led to believe that this was a case of direct reduction of nitrates to ammonia. It seemed probable, however, that the ammonia was the product of the breaking down of the peptone present in the nitrate broth. This assumption was carefully tested and proved to be correct. The results of this study were reported at the September meeting of the American Public Health Association, before the Laboratory Section. None of the organisms tested seemed to possess the power of breaking down nitrates to ammonia in a nitrate-peptone solution, the ammonia being derived entirely from the peptone. The production of ammonia from peptone proved to be a distinct and valuable test.

9. *Production of ammonia* in nitrate-free peptone broth was tested by inoculating each organism into five tubes containing a solution of 1 per cent peptone in distilled water to which some inorganic salts were added. These tubes were incubated for seven days at 20° C. The presence of ammonia was tested in the usual way by Nesslerization and the results were recorded as positive or negative. The value of this test is brought out by a correlation of the pigment-producing property with that of ammonification of peptone as shown in Table 1.

<sup>1</sup> *Systematic Relationship of the Coccaceae*, 1908.

TABLE 1.  
CORRELATION BETWEEN CHROMOGENESIS, REDUCTION OF NITRATE, AND AMMONIFICATION  
OF PEPTONE

CHROMOGENESIS	TOTAL NUM- BER OF STRAINS	REDUCTION OF NITRATE TO NITRITE		SPLITTING OF PEPTONE TO AMMONIA	
		Number	Percentage	Number	Percentage
None.....	6	0	0	0	0
White.....	12	1	8.3	4	33.3
Orange*.....	15	11	73.3	14	93.3
Yellow.....	15	2	13.3	12	80.0
Red.....	5	5	100.0	1	20

\* Including the orange sarcinae.

10. *Comparative growth* after 14 days at 20° and 37° respectively. Recorded as: (a) better at 37°; (b) same; (c) poorer at 37°.

11. *Chromogenesis*.—The color of the pigment produced on agar streaks, after 14 days at 20° C. A loopful of the growth was spread in a thin uniform layer on white paper and dried at room temperature. This was then compared with the chart given as the frontispiece of the Winslows' book on the Coccaceae.<sup>1</sup> It is essential that an approximately equal amount of growth be used and that this be spread evenly on the paper. This is especially important in the case of the meager-growing orange and white cocci. In these organisms the pigments, when observed on the brownish agar background, can very readily be confused. As will be seen from the table below, at least one typical aurococcus (No. 218) was sent to us as an albococcus. The distinction is quite sharp, when the growth is spread and examined on paper. The chromes, using the chart in the Winslows' book, fall as follows:

*White*.—white column; light lemon yellow I–III; cadmium yellow I–III.

*Yellow*.—cadmium yellow IV–IX; medium cadmium yellow column; orange yellow I–IV.

*Orange*.—orange yellow V–IX; cadmium orange column.

*Red*.—orange red; medium red, dark red.

12. *Gelatin liquefaction*.—Recorded in centimeters after 30 days at 20° C., in tubes about 1.1 cm. in diameter.

<sup>1</sup> *Op. cit.*

TABLE 2.

No. of Culture	Source	Original Name	Grouping of Cells	Dimension in $\mu$	Gram Reaction	Growth after 14 Days at		Acid Production Percentage Normal in			Reduction of Nitrates to Nitrites	Splitting of Peptone to Ammonia	Liquefaction of Gelatin No. of cms.	Chromo-Genesis	New Name
						20° C.	37° C.	Dext.	Lact.	Sac.					
3..	U. of Penn. Rockefeller Inst.	<i>S. aurantiaca</i>	Packets	0.9	—	Abundant	Poorer	1.4	—0.9	—0.4	—	+	2.6	Orange	<i>S. aurantiaca</i>
4..	From arm vein, U. of C.	<i>M. melitensis</i>	No packets	0.7	—	Meager	Better	2.0	2.8	4.4	—	+	2.7	Orange	<i>Aur. mollis</i>
*33..			"	0.4		Meager	Same	—0.1	—0.2	0.0	—	+	0.0	Orange	<i>B. melitensis</i>
34..	Rockefeller Inst.	<i>M. rheumaticus</i>	"	0.8	—	Faint	Same	3.6	2.8	0.5	—	—	0.0	None	<i>Str. rheumaticus</i>
101..	Lab. air, Del. Ag. Col.	<i>Sarcina</i>	Packets	0.8	—	Abundant	Same	0.0	0.0	0.3	—	+	1.4	Yellow	<i>S. flava</i>
113..	Air contamination, U. of Pa.	<i>S. flava</i>	"	0.6	—	"	Poorer	1.2	—0.3	1.2	—	+	2.2	Yellow	<i>S. flava</i> (Var. B)
128..	Cesspool, U. of C.	<i>M. zymogenes</i>	No packets	0.8	—	Faint	Same	6.0	2.8	0.7	—	—	4.5	None	<i>Str. gracilis</i>
130..	Urine, Mt. Prospect Lab.	<i>M. ureae</i>	"	0.6	++	Good	Poorer	1.9	2.0	2.7	—	+	1.1	White	<i>Alb. ureae</i>
174..	U. of C.	<i>S. capsulata</i>	"	0.6	++	Good	Same	2.1	2.8	3.1	—	+	0.0	White	<i>Alb. candidus</i>
207..	Subcutaneous abscess, U. of Iowa	"	"	0.6	±	Meager	Better	3.0	2.8	3.5	—	+	4.7	Orange	<i>Aur. mollis</i>
208..	Stomach contents of chronic dyspepsia, U. of Iowa	<i>S. lutea</i>	Packets	0.6	±	Very abundant	Poorer	0.3	0.0	0.3	—	+	2.8	Yellow	<i>S. flava</i> (Var. A)
209..	Abscess in calf of leg, U. of Iowa	<i>M. tetragenus</i>	Tetrads	0.6	±	Meager	Same	1.9	3.0	2.3	—	—	0.0	White	<i>Alb. tetragenus</i>
216..	Tap water, J.H.U.	<i>S. lutea</i>	Packets	0.7	+	Very abundant	Poorer	1.6	0.3	1.0	—	+	4.7	Yellow	<i>S. flava</i> (Var. B)
217..	J.H.U.	<i>Albococcus</i>	No packets	0.8	—	Abundant	Same	0.5	—0.2	0.0	—	—	0.0	Red	<i>Rh. roseus</i>
218..	J.H.U.	"	"	0.8	—	Meager	Same	2.0	1.0	2.4	—	+	4.7	Orange	<i>Aur. mollis</i>
219..	J.H.U.	"	"	0.7	+	Meager	Better	2.8	2.0	4.3	—	+	4.2	Orange	<i>Aur. mollis</i>
220..	J.H.U.	<i>M. cereus</i>	"	0.7	+	Abundant	Poorer	—0.5	—0.8	—0.3	—	—	0.0	Yellow	<i>M. luteus</i>
260..	P.D. & Co.	<i>M. neoformans</i>	"	0.7	+	Good	Same	1.8	—0.4	0.0	—	—	0.0	White	<i>Alb. candidans</i>
261..	Abscess of horse, P.D. & Co.	<i>St. pyogenes</i>	"	0.7	—	Good	Same	1.2	—0.3	0.6	—	—	0.0	White	<i>Alb. candidans</i>
262..	Abscess of horse, P.D. & Co.	<i>St. pyogenes</i>	"	0.6	±	Meager	Same	2.7	2.0	2.6	—	+	2.2	Orange	<i>Aur. mollis</i>
263..	Skin lesion, P.D. & Co.	<i>St. pyog. albus</i>	"	0.8	+	Meager	Better	1.8	1.7	2.7	—	—	1.0	White	<i>Alb. pyogenes</i>
264..	Bolls, P.D. & Co.	<i>St. pyog. aureus</i>	"	0.7	±	Meager	Better	2.5	2.8	4.0	—	+	3.2	Orange	<i>Aur. mollis</i>
259..	P.D. & Co.	<i>M. rheumaticus</i>	"	0.8	—	Faint	Same	6.8	4.3	7.2	—	—	0.0	None	<i>Str. rheumaticus</i>
272..	P.D. & Co. (Kral)	<i>St. cereus flavus</i>	"	0.7	—	Very abundant	Poorer	0.0	0.4	0.8	—	—	2.3	Yellow	<i>M. flavus</i>
279..	Acne vulgaris, P.D. & Co.	<i>St. pyogenes citreus</i>	"	0.7	+	Meager	Better	1.5	2.5	3.8	—	+	4.3	Orange	<i>Aur. mollis</i>
312..	Mammary abscess, Boston Board of Health	"	"	0.7	±	Meager	Better	4.0	2.2	3.2	—	+	2.8	Orange	<i>Aur. mollis</i>

\* Repeated stains of cultures of different ages showed this to be a small bacillus, .4X.5  $\mu$ .

TABLE 2—Continued.

313..	Pus. B.B. of H.	S. lutea	"	Packets	"	0.8	+	Meager	Same	1.9	3.6	3.8	+	+	1.9	Orange	Aur. mollis (Var. A)
314..	B.B. of H.	S. aurantiaca	"	"	"	0.8	+	Abundant	Poorer	0.0	0.0	0.3	+	+	1.4	Yellow	S. flava (Var. A)
315..	B.B. of H.	S. lutea	"	"	"	0.6	+	Very abundant	Poorer	2.1	-1.0	0.3	+	+	2.6	Orange	S. aurantiaca
345..	Mich. Agri. Col.	S. lutea	"	"	"	0.6	+	Very abundant	Poorer	0.6	0.4	0.1	+	+	1.2	Yellow	S. flava
347..	Cow. Mich. Agri. Col.	St. pyogenes	"	No packets	"	0.7	+	Meager	Better	2.5	1.8	3.6	+	+	3.4	Orange	Aur. pyogenes
348..	Mich. Agri. Col.	St. pyogenes	"	"	"	0.8	+	Good	Poorer	2.3	1.8	3.8	+	+	5.2	Orange	Aur. mollis
354..	Butter, Mich. Agri. Col.	M. citreus	"	Tendency to packets	"	0.9	+	Very abundant	Poorer	2.2	0.4	0.0	+	+	1.2	Yellow	M. flavus
399..	Articular rheumatism, Hoagland Laboratory	M. rheumaticus	"	No packets	"	0.8	+	Faint	Same	4.4	4.6	8.6	+	+	0.0	None	Str. rheumaticus
430..	U. of Pa.	M. versatilis	"	Irregular tetrads	"	0.6	+	Abundant	Poorer	0.0	-0.2	0.6	+	+	0.0	Yellow	M. luteus
437..	Nasal secretion, U. of Pa.	M. catarrhalis	"	No packets	"	0.7	+	Faint	Same	3.5	2.3	6.6	+	+	0.0	None	Streptococcus
457..	Kral	M. ascoformans	"	"	"	0.6	+	Meager	Better	1.6	3.0	3.6	+	+	1.9	Orange	Aur. mollis
458..	Kral	M. citreus	"	"	"	0.9	+	Abundant	Poorer	0.0	0.0	-0.7	+	+	5.1	Yellow	M. flavus
462..	Kral	S. rosea	"	some tetrads	"	0.7	+	Good	Poorer	0.8	0.3	-0.2	+	+	0.0	Red	Rh. roseus
464..	Kral	M. ureae	"	"	"	0.6	+	Good	Same	1.9	1.5	3.3	+	+	2.2	White	Alb. ureae
466..	Kral	M. agilis	"	"	"	0.6	+	Abundant	Poorer	0.0	-0.2	-0.6	+	+	1.1	Yellow	M. citreus
467..	Kral	M. aurantiacus	"	"	"	0.9	+	Good	Same	2.5	1.5	1.3	+	+	0.0	White	Alb. epidermidis (Var. A)
471..	Kral	M. candidans	"	No packets	"	0.7	+	Good	Poorer	1.7	1.5	2.6	+	+	0.0	White	Alb. candidans
474..	"	S. aurantiaca	"	"	"	0.9	+	Abundant	Same	2.9	1.0	-0.4	+	+	3.5	Orange	S. aurantiaca
476..	"	"	"	"	"	0.8	+	Faint	Same	5.0	5.0	7.0	+	+	0.0	None	Streptococcus
479..	"	S. ventricula	"	"	"	0.9	+	Abundant	Poorer	0.0	-0.2	-0.2	+	+	0.0	Yellow	M. citreus (Var. A)
480..	"	M. rhodochrous	"	"	"	0.6	+	Good	"	0.0	-0.0	-0.1	+	+	0.0	Red	Rh. roseus
481..	"	S. flava	"	"	"	0.8	+	Very abundant	"	0.0	0.0	0.0	+	+	1.7	Yellow	M. flavus
482..	"	S. mobilis	"	Packets	"	0.9	+	Very abundant	"	0.0	-0.4	0.2	+	+	2.4	Yellow	S. flava (Var. A)
483..	"	M. roseus	"	No packets	"	0.9	+	Good	"	0.9	-0.7	0.9	+	+	0.0	Red	Rh. roseus
484..	"	M. tetragenus	"	Tetrads	"	0.6	+	Good	Better	2.6	2.8	4.4	+	+	0.0	White	Alb. tetragenus
488..	"	M. agilis	"	No packets	"	0.9	+	Good	Poorer	0.3	-0.7	0.0	+	+	0.9	Red	Rh. roseus (Var. A)
525..	P.D. Co. acne-like ulcer	M. acne	"	"	"	0.7	+	Meager	Better	2.6	1.6	2.1	+	+	0.0	White	Alb. candidans
526..	U. of Wis.	M. neoformans	"	"	"	0.7	+	Good	Same	1.9	0.6	2.1	+	+	0.0	White	Alb. candidans

A complete record of all the strains studied, giving in detail the history of each culture (as far as possible), the original name under which it was received, the various tests recorded as observed, and finally the new name according to the Winslow classification, is shown in Table 2. This table points out very clearly the necessity of a definite system of classification based on real differences and definite properties. We see here a list of organisms, some of which (like 217, 462, and 483), differ in nothing but in the name under which they were sent to us; others (like 218 and 279) are not really what they were supposed to be. This can be attributed to nothing else than the poverty of the traditional descriptions, inevitable under a system of differentiation based on such variable factors as surface growth on various media, types and contour of colonies, etc.

On breaking up this table and grouping the organisms according to the general correlation of their properties, we obtain five main groups agreeing in all essentials with the genera defined by the Winslows. Table 4 shows a group of non-pigment producing, high acid forming, faintly growing organisms. These belong undoubtedly to the genera *Streptococcus* and *Diplococcus* as defined by the Winslows. The white-pigment producers grouped in Table 5 answer well to the characteristics of the genus *Albococcus* (Winslow and Rogers). The essential characters of the group, such as generally gram-positive, good growth on agar, moderate acid production in all three sugars, slight gelatin liquefaction and nitrate reduction, correspond with those attributed to this genus.

The orange cocci, as seen from Table 6, also present, in accordance with the findings of the Winslows, a distinct and definite picture. These organisms generally stain by Gram, give a meager growth on agar, ferment all the sugars tested, actively liquefy gelatin, and generally reduce nitrates. This group agrees very well with the genus *Aurococcus* (Winslow and Rogers).

In Table 7 (*a* and *b*) are included all the strains producing yellow pigment. This table brings out very strikingly the fact that there is practically no difference between the yellow micrococci and sarcinae other than that of cell grouping. The value of packet formation as a generic differential seems extremely doubtful. In accord-

ance with the Winslows, however, this group is divided into two parts: (a) corresponding to the genus *Micrococcus* (Hallier, Cohn) and (b) to the genus *Sarcina* (Goodsir). The group as a whole presents a definite unit and agrees well with the Winslows' definition. It generally decolorizes by Gram; gives abundant growth on agar; slight acid in dextrose; a generally neutral reaction in lactose; while gelatin is frequently liquefied.

The last group, consisting of the red-pigment producers, is shown in Table 8. Like the others, it corresponds very closely to its respective genus *Rhodococcus* (Winslow and Rogers). It is generally gram-negative and gives good to abundant surface growth. Sugars are but slightly fermented. Gelatin is rarely liquefied. Nitrates are generally reduced.

TABLE 3.  
CORRELATION OF CHARACTERS OF THE DIFFERENT GENERA.

CHROMO- GENESIS	GENUS	GROUPING OF CELLS; PERCENTAGE PACKETS	GRAM REACTION; PERCENTAGE NEGATIVE	SURFACE GROWTH; PERCENTAGE GIVING				ACIDITY; PERCENTAGE ABOVE 0.01 N.			NITRATE REDUCTION; PERCENTAGE REDUCERS	PEPTONE AMMONIFICATION; PERCENTAGE AMMONIFIERS	GELATIN LIQUEFACTION; PERCENTAGE LIQUEFIERS	GELATIN LIQUEFACTION; AVERAGE IN CM.
				Faint	Meager	Good	Abundant	Dextrose	Lactose	Saccharose				
None.....	Streptococcus and Diplo- coccus	0	66	100	0	0	0	100	100	66	0	0	17	...
White.....	Albococcus	0	17	0	25	75	0	100	75	85	9	33	25	1.4
Orange*.....	Aurococcus	20	7	0	73	7	20	100	80	80	73	93	87	3.5
Yellow.....	Micrococcus	0	87	0	0	0	100	12	0	0	25	87	63	2.2
Yellow.....	Sarcina	100	71	0	0	0	100	29	0	0	0	71	100	2.3
Red.....	Rhodococcus	0	80	0	0	80	20	0	0	0	100	20	20	0.9

\* Includes orange sarcinae.

The generic classification proposed by the Winslows is thus found to apply easily and satisfactorily to the 54 strains of cocci in our collection. While there may be now and then an aberrant strain, the remarkable general correlation between pigment production and other properties justifies the grouping of the cocci into the genera named above.

A summary of the correlation of the various characters of the different generic groups is given in Table 3. The comparatively small number of organisms in each group leads often to percentages

which are either too high or too low; and it is of course obvious that the classification is not a sharp nor absolute one. For instance, the yellow sarcinae are characteristically gram-negative, yet 29 per cent of the strains studied showed a positive reaction. Bacteria are exceedingly variable organisms and this variability is indeed the main reason for the use of the biometric method. The general agreement which exists between the results tabulated below and those obtained in Boston<sup>1</sup> five years ago is too close to be accounted for except by an inherent, though not infallible, correlation between the various properties considered; and such a correlation can only be explained on a basis of genetic relationships.

On analyzing each of the main groups carefully we find that the classification of species proposed by the Winslows is not quite as satisfactory as their generic grouping. There appear to be certain forms which the authors have not found among the organisms studied by them but which are important enough to deserve specific rank. This is exactly what might have been expected and can be remedied only by further and more elaborate study. The behavior of the cocci in splitting up peptone, which is here used for the first time, of course, increases the possibility of defining new and distinct species.

Of the strains grouped in Table 4, No. 128 undoubtedly belongs to the species *Str. gracilis* of which *M. zymogenes* is a synonym. Nos. 34, 259, and 399, sent to us as *M. rheumaticus*, may for the present be retained as *Str. rheumaticus*, since their behavior (faint growth, high acid production, and negative reaction in other media) corresponds to that of the genus *Streptococcus*, while they have nothing in common with the micrococci. Whether this group is distinct enough to deserve specific rank or not is still an open question to be decided by a comparative study of a large number of strains of this type. In a recent paper Major<sup>2</sup> has reported a more detailed examination of the fermentation reactions of these three strains and finds that No. 34 belongs to the *S. salivarius* while Nos. 259 and 399 are examples of the *S. fecalis* of Andrewes and Horder. No. 437 was sent to us as *M. catarrhalis*. This coccus is defined

<sup>1</sup> Winslow, C.-E. A and A. R., *Systematic Relationship of the Coccaceae*.

<sup>2</sup> *Johns Hopkins Hosp. Bull.*, 1912, 23, p. 326.

TABLE 4.  
No PIGMENT PRODUCERS

No. OF CULTURE	ORIGINAL NAME	GROUPING OF CELLS	DIMENSION IN $\mu$	GRAM REACTION	GROWTH AT		ACID PRODUCTION IN			NITRATE REDUC-TION TO NO <sub>2</sub>	BREAKING DOWN OF PEPTONE TO NH <sub>3</sub>	GELATIN LIQUE-FACTION	NEW NAME
					20° C.	37° C.	Dext.	Lact.	Sacch.				
128.....	M. zymogenes	Short chains	0.8 "	-	Faint	Same	+	+	-	-	-	+	Str. gracilis
34.....	M. rheumaticus	Irregular groups and small chains	0.8 "	-	"	"	+	+	-	-	-	-	Str. rheumaticus
259.....	M. rheumaticus	Irregular groups, short chains	0.8 "	-	"	"	+	+	+	-	-	-	"
399.....	M. rheumaticus	Irregular groups, short chains	0.8 "	-	"	"	+	+	+	-	-	-	"
437.....	M. catarrhalis	Short chains	0.7 "	+	"	"	+	+	+	-	-	-	Str. ?
476.....	?	Irregular groups, short chains	0.8 "	-	"	"	+	+	+	-	-	-	Str. ?

by Frosh and Kolle<sup>1</sup> as gram-negative, and was found by Elser and Huntoon<sup>2</sup> to be gram-negative and non-fermenting. Our strain is gram-positive and ferments sugars actively. It cannot, therefore, belong to this species. Its actual place, as well as that of No. 476, must be left open for the present, though both of them undoubtedly belong to the genus *Streptococcus*.

No attempt has been made to include the large number of streptococci in the Museum collection in this study. The six forms mentioned above came to us as micrococci and were classed as such until this examination was made. This illustrates clearly how, under the present system of classification, totally unrelated organisms are included in the same genus.

In the white-pigment producing group (see Table 5) we find that No. 263 corresponds to the species *Alb. pyogenes* (Rosenbach) Winslow, in that it liquefies gelatin and does not reduce nitrates. Nos. 464 and 130 are, like 263, liquefiers and non-reducers, but unlike that strain they possess the power of breaking down peptone to ammonia. The Winslows do not recognize this as a distinct species, yet those two organisms differ from the nearest type (*Alb. pyogenes*), in the important properties of growth abundance and formation of ammonia from peptone. Both organisms were sent to us as *M. urea*, and urea-fermenting organisms having the power of producing ammonia are described by many observers. Flügge<sup>3</sup> defines a white, urea-fermenting, gelatin-liquefying coccus as *M. Ureae-liquefaciens*. It seems justifiable, therefore, to recognize this type and characterize it as follows: *Alb. ureae* (Cohn, Flügge), a white coccus occurring singly or in irregular groups, generally found in the human or animal body. Generally gram-positive. Good surface growth. Produces moderate acid in dextrose, saccharose, and lactose media. Gelatin liquefied. Nitrate not reduced. Peptone decomposed to ammonia.

Nos. 484 and 209 (Table 5) evidently belong to the species *Alb. tetragenus* (Cohn) Winslow. This type is a non-reducer and a non-liquefier and possesses the characteristic grouping of cells in

<sup>1</sup> *Die Mikrokokken*. Flügge's *Die Mikroorganismen*, 1896, Vol. 2.

<sup>2</sup> *Jour. Med. Research*, 1909, N. S. 15, p. 413 and p. 427.

<sup>3</sup> *Die Mikroorganismen*, 1896, Vol. 2, p. 173.

TABLE 5.  
WHITE PIGMENT PRODUCERS.

No. OF CULTURE	ORIGINAL SOURCE	ORIGINAL NAME	GROUPING OF CELLS	DIMENSION IN $\mu$	GRAM REACTION	GROWTH AT		ACID PRODUCTION IN			BREAKING DOWN OF PEPTONE TO $\text{NH}_3$	REDUCTION OF NITRATE TO $\text{NO}_2$	GELATIN LIQUEFACTION	NEW NAME
						20° C.	37° C.	Dext.	Lact.	Sacch.				
263..	Pathogenic	St. pyogenes albus	No packets	0.8	+	Meager	Better	+	+	+	+	+	+	Alb. pyogenes
130..	Human	M. ureae	"	0.6	++	Good	Poorer	+	+	+	+	+	+	Alb. ureae
404..	"	"	"	0.6	++	Good	Same	+	+	+	+	+	+	Alb. ureae
409..	Pathogenic	M. tetragenus	tetrads	0.6	++	Good	Same	+	+	+	+	+	+	Alb. tetragenus
484..	"	"	"	0.6	++	Good	Better	+	+	+	+	+	+	"
474..	Pathogenic	S. capsulata	No packets	0.6	++	Good	Same	+	+	+	+	+	+	Alb. candidus
471..	"	M. candidans	"	0.7	++	Good	Same	+	+	+	+	+	+	"
325..	Pathogenic	M. acne	"	0.7	++	Meager	Poorer	+	+	+	+	+	+	"
407..	"	M. aurantiacus	"	0.9	++	Good	Same	+	+	+	+	+	+	Alb. epidermidis (Var. A)
260..	"	M. neoformans	"	0.7	++	Good	Same	+	+	+	+	+	+	Alb. candidans
261..	Horse abscess	St. pyog. albus	"	0.7	++	Good	Same	+	+	+	+	+	+	"
326..	"	M. neoformans	"	0.7	++	Good	Same	+	+	+	+	+	+	"

tetrads. Nos. 174, 471, and 525 agree with the definition of *Alb. candidus* (Cohn) Winslow, in failing to reduce nitrates or liquefy gelatin. No. 174 came to us as a sarcina which it is not. No. 467 came to us as *M. aurantiacus*, but is not an orange-pigment former. It apparently finds no place in the Winslows' classification. The authors call this a rare aberrant type, which may be termed a "variant by suppression" of the reducing and liquefying type which has lost the latter property. Though this one strain does not justify the creation of a new type center, yet the recurrence of similar *non-liquefying* and *reducing* types in the other groups tends to point to the possible existence of such a type center. Temporarily it may be called *Alb. epidermidis* (Var. A). Nos. 260, 261, and 526 are even more puzzling. These seem to occupy a position between the albococci and micrococci. They differ from the albococci mainly in their lack of power to ferment the disaccharids. Winslow mentions this species as the second of Gordon's four types of skin cocci and places it among the micrococci as *M. candicans*. From the work of Gordon it appears, however, that they are body forms. Their relation to the albococci in gram reaction, pigment production, growth abundance and fermentation of dextrose is closer than to the micrococci. It appears, therefore, more consistent to place them in this group. This question may perhaps best be left open until more work has been done; but for the present I suggest the name *Alb. candicans* rather than *M. candicans*, for the type of white coccus which acidifies dextrose but not lactose and fails to reduce nitrates or liquefy gelatin. It is interesting to note that two of these three cultures were sent to us under the name *M. neoformans*, the coccus isolated from cancerous tissue by Doyen.

Examining the third group (see Table 6), we find that No. 347 in liquefying gelatin and not reducing nitrates answers to the type *Aur. aureus* (Rosenbach) Winslow. *Aur. mollis* (Dyar) Winslow, the reducing and liquefying type, is represented in our collection by Nos. 218, 219, 262, 264, 4, 207, 279, 312, 348. Nos. 313 and 457, reducers but slow liquefiers, are aberrant types similar to No. 467 among the albococci. For similar reasons these may be classed as a variety of the reducing and liquefying species *Aur.*

TABLE 6.  
ORANGE PIGMENT PRODUCERS.

No. of Culture	Original Source	Original Name	Grouping of Cells	Dimension in $\mu$	Gram Reaction	Growth at		Acid Production in			Splitting of Peptone to NH <sub>3</sub>	Reduction of Nitrate to NO <sub>2</sub>	Gelatin Liquefaction	New Name
						20° C.	37° C.	Dext.	Lact.	Sacch.				
347...	Parasitic	St. pyogenes	No packets	0.7	±	Meager	Better	+	+	+	+	+	Aur. aureus	
4...	"	"	"	0.7	±	"	"	+	+	+	+	+	Aur. mollis	
207...	Parasitic	"	"	0.6	±	"	"	+	+	+	+	+	"	
262...	"	St. pyogenes aureus	"	0.6	±	"	Same	+	+	+	+	+	"	
218...	"	Albococcus	"	0.7	±	"	Better	+	+	+	+	+	"	
219...	"	"	"	0.7	±	"	"	+	+	+	+	+	"	
264...	Parasitic	St. pyogenes aureus	"	0.7	±	"	"	+	+	+	+	+	"	
279...	"	St. pyogenes citreus	"	0.7	±	"	"	+	+	+	+	+	"	
312...	"	"	"	0.7	±	"	"	+	+	+	+	+	"	
348...	"	St. pyogenes	"	0.8	±	Good	Little	+	+	+	+	+	"	
313...	"	"	"	0.8	±	Meager	Poorer	+	+	+	+	+	Aur. Mollis (Var. A)	
457...	Parasitic	M. ascoformans	"	0.6	±	"	Same	+	+	+	+	+	"	
3...	"	S. aurantiaca	Packets	0.9	—	Abundant	Better	+	+	+	+	+	Sarcina aurantiaca	
315...	"	"	"	0.8	±	"	Poorer	+	+	+	+	+	"	
474...	"	"	"	0.9	±	"	"	+	+	+	+	+	"	

*mollis*. It may be noted that No. 457 came to us under the name *M. ascoformans*.

Strains 3, 315, and 474, though producing a strong orange color, present a picture entirely different from that of the aurococci. The generally abundant growth, frequent decolorization by Gram (more often than aurococci), better growth at 20°, lack of fermentative power of lactose and saccharose, slower liquefaction of gelatin, together with the formation of packets, bring this type closer to the yellow sarcinae than to the aurococci. It differs from the yellow sarcinae, however, in its orange pigment and in attacking dextrose. The Winslows state that the 11 strains of orange-packet formers with which they worked were generally gram-positive, acid-forming, actively liquefying forms, and they are inclined to refer them to the genus *Aurococcus*. The three strains here studied seem, however, to be much more closely related to the genus *Sarcina* and will be considered further under that head.

Another form studied in this group was No. 33, the *M. melitensis* of Bruce. It is a meager, gram-negative, orange-pigment former, giving negative reactions in all media. A careful examination of our strain, however, confirms the conclusion of Babes<sup>†</sup> that this organism is a small bacillus.

Examining the micrococcus group shown in Table 7a, No. 466 represents the liquefying, reducing type defined as *M. citreus* (Dyar) Winslow. No. 479 in its failure to liquefy gelatin represents another of the aberrant types discussed before and may be classed as variety A of *M. citreus*. This organism came to us as a sarcina. Though no packets could be observed, its cultural characters make it appear probable that it is related to the *S. ventriculi* of Goodsir. Nos. 272, 354, 458, and 481 belong to the non-reducing, liquefying type *M. flavus* (Flügge) Migula. This type seems also to be characterized by the property of splitting peptone to ammonia. Whether this character is specific or not can be brought out only by further comparative study of the behavior of the cocci in this respect. No. 430 is grouped with 220 as *M. luteus* (Cohn) Migula, the non-reducing, non-liquefying type, although it differs from it in breaking down peptone to ammonia.

<sup>†</sup> Kolle and Wasseman, *Handbuch der pathogenen Mikroorganismen*, 1903, 3, p. 438.

TABLE 7.  
YELLOW PIGMENT PRODUCERS.  
(a) NON-PACKET FORMERS.

No. of Culture	ORIGINAL SOURCE	ORIGINAL NAME	GROUPING OF CELLS	DIMENSION IN $\mu$	GRAM REACTION	GROWTH AT		ACID PRODUCTION IN			BREAKING DOWN OF PEPTONE TO $\text{NH}_3$	REDUCTION OF NITRATE TO $\text{NO}_2$	GELATIN LIQUEFACTION	NEW NAME
						20° C.	37° C.	Dext.	Lact.	Sacch.				
466....	.....	M. agilis (citreus)	No packets	0.6	—	Abundant	Poorer	—	—	—	±	—	+	M. citreus
470....	.....	S. ventricula	" "	0.9	—	Very abundant	"	—	—	—	±	+	+	" (Var. A)
272....	.....	Staph. cereus flavus	" "	0.7	—	Abundant	"	+	—	—	+	—	+	M. flavus
354....	Butter	M. citreus	"	0.9	—	Abundant	"	—	—	—	+	—	+	"
458....	.....	"	Some tetrads, No packets, some tetrads	0.9	—	"	"	—	—	—	+	—	+	"
481....	.....	S. flava	No packets	0.8	—	Very abundant	"	—	—	—	+	—	+	"
220....	.....	M. cereus	"	0.7	—	Abundant	"	—	—	—	+	—	+	M. luteus
430....	.....	M. versatilis	No packets, some tetrads	0.6	—	Abundant	"	—	—	—	+	—	+	"

(b) PACKET FORMERS.

101....	Air	Sarcina	Packets	0.8	—	Abundant	Same	—	—	—	+	—	+	S. flava
345....	.....	S. lutea	"	0.6	—	Very abundant	Poorer	—	—	—	+	—	+	" (Var. A)
208....	Stomach content	"	"	0.6	—	"	"	—	—	—	+	—	+	"
314....	.....	S. mobilis	"	0.8	±	Abundant	"	—	—	—	+	—	+	"
482....	.....	S. flava	"	0.9	—	"	"	—	—	—	+	—	+	"
113....	Air	S. lutea	"	0.6	—	Very abundant	"	—	—	—	+	—	+	" (Var. B)
210....	Water	"	"	0.7	+	"	"	+	+	+	+	—	+	"
3....	.....	S. aurantiaca	"	0.9	—	Abundant	"	+	+	+	+	—	+	S. aurantiaca
315....	.....	"	"	0.9	±	"	"	+	+	+	+	—	+	"
474....	.....	"	"	0.8	±	"	"	+	+	+	+	—	+	"

In Table 7b—the *Sarcina* group—101 and 345 belong to the liquefiers and non-reducers, which are classed under the type *S. flava* (De Bary). Nos. 113, 216, 208, 314, 482 are all similar to *S. flava* in that they do not reduce nitrates but liquefy gelatin. They all differ from that form in their power to break up peptone to ammonia. Though not justified in recognizing a new type center, yet the comparatively large percentage of our sarcinae which fall in this group indicates at least the possible existence of such a species. Temporarily, they may be classed, therefore, as a variety of *S. flava*. No. 482 came to us as *S. mobilis*, supposed to be a motile red-pigment former, which it is not. Two forms, 113 and 216, of this subgroup show a further remarkable distinction in being able to ferment dextrose and saccharose. This points to a close relationship between these forms and the orange sarcinae. It is highly probable that these are intermediary forms between the yellow and orange sarcinae and possibly between the micrococci and the aurococci. If these forms occur frequently they certainly deserve specific recognition. Here again, however, two strains do not justify the establishing of a new type center. They are, therefore, for the present, classed as another variety (Var. B) of *S. flava*. A further study of this whole group is necessary and highly desirable.

Nos. 3, 315, and 474 remain to be considered. These are the orange sarcinae, which, as pointed out above, seem more nearly allied to the yellow sarcinae than to the aurococci. These three strains were all sent to us as *S. aurantiaca*, and all liquefy, corresponding with Flügge's description of that species. The type may therefore bear the name commonly given to it with the following definition:

*S. aurantiaca* (Flügge).—A large saprophytic coccus. Occurs in packets. Variably affected by Gram. Abundant orange growth. Reaction in dextrose broth moderately acid, in lactose and saccharose faintly alkaline. Gelatin liquefied. Nitrates not reduced. Peptone ammonified.

The definition of the species of the last genus proposed by the Winslows, *Rhodococcus*, is not quite satisfactory. This is undoubtedly due to the small number of strains studied by the authors. According to their classification all the strains grouped in Table 8

TABLE 8.  
RED PIGMENT PRODUCERS.

No. of Culture	Original Source	Original Name	Grouping of Cells	Dimension in $\mu$	Gram Reaction	Growth at		Acid Production in			Breaking Down of Peptone to $\text{NH}_3$	Reduction of Nitrate to $\text{NO}_2$	Gelatin Liquefaction	New Name
						20° C.	37° C.	Dext.	Lact.	Sacch.				
217.....	.....	.....	No packets	0.8	+++	Abundant	Same	—	—	—	++++	++++	++++	Rhod. roseus
462.....	.....	Sarcina rosea	"	0.7	+++	"	Poorer	—	—	—	++++	++++	++++	"
480.....	.....	M. rhodochrous	"	0.6	+++	"	"	—	—	—	++++	++++	++++	"
483.....	.....	M. roseus	"	0.9	+++	"	"	—	—	—	++++	++++	++++	"
488.....	.....	M. agilis	"	0.9	+++	"	"	—	—	—	++++	++++	++++	R. roseus (Var. A)

belong to the species *R. roseus* (Flügge, Dyar) Winslow. Strains 217, 462, and 483 are undoubtedly representatives of this species. No. 480 possibly also belongs here, though it was the only form that gave repeatedly a gram-positive reaction. No. 488, however, appears to be out of place in this group. Its pigment is of a deeper hue than that of any of the others (Nos. 217, 463, and 483 all fall in the first three [lighter] hues of the orange red of the Winslows' chart, while No. 488 falls in hue 5 of the dark red); it liquefies gelatin and shows a tendency to break up peptone to ammonia. It is as different from the others as one species can be from another. It may also be observed that this organism was sent to us as *M. agilis*. No motility could be observed however. The type is not defined by the Winslows, and one strain hardly justifies such a definition. We must resort again, therefore, to the distinction by variety and call this form *R. roseus* Var. A.

#### SUMMARY AND CONCLUSIONS.

The 54 strains of the cocci in the American Museum collection group themselves very definitely according to pigment production and other characters into five distinct classes. The correlation of the various morphological and biochemical properties bears out the work by the Winslows and justifies their recognition of five genera among the Coccaceae outside of the diplococci and streptococci.

Six of the strains give faint growth on agar, produce no pigment, ferment sugars very actively and belong, therefore, to the genera *Diplococcus* and *Streptococcus*. Twelve strains produce white pigment, good growth, moderate acidity, are generally gram-positive, liquefy gelatin slowly, if at all, and conform in every way with the genus *Albococcus*. There are three exceptions in this group, which produce no acidity in lactose and saccharose and probably form the connecting link between the true albococci and micrococci.

Orange-pigment producers number 15. This group is distinguished from the white by meager growth, reduction of nitrates, and rapid liquefaction of gelatin. It agrees perfectly with the genus *Aurococcus*. Three strains are packet formers and in other prop-

TABLE 9.  
TABULAR KEY TO THE GENERA AND SPECIES OF COCCACEAE

GENUS	SPECIES	VARI- ETY	SOURCE	CELL GROUPING	DIMENSION AVER- AGE IN $\mu$	GRAM GENERALITY	GROWTH AT		ACID PRODUCTION IN			BREAKING DOWN OF PEPTONE TO $\text{NH}_3$	REDUCTION OF NITRATE TO $\text{NO}_2$	LIQUEFACTION OF GELATIN	CHROMO- GENESIS
							20° C.	37° C.	Dext.	Lact.	Sacch.				
Str. Alb. Paracoccaceae	Gracilis.....	....	Parasitic	No packets	0.8	—	Faint	Same	++	++	—	—	—	—	None
	Rheumaticus.....	....	"	"	0.8	—	Meager	Better	++	++	—	—	—	—	None
	Pyogenes.....	....	"	"	0.8	—	Good	Same	++	++	—	—	—	—	White
	Ureæ.....	....	"	"	0.6	—	Good	Better	++	++	—	—	—	—	White
	Tetragenus.....	....	"	Tetrads	0.6	++	Good	Same	++	++	—	—	—	—	White
	Candidus.....	....	"	No packets	0.6-0.7	++	Good	Same	++	++	—	—	—	—	White
	Epidermidis.....	....	"	"	..	—	Good	Same	++	++	—	—	—	—	White
	Epidermidis.....	A	"	"	0.9	—	Good	Same	++	++	—	—	—	—	White
	Candicans.....	....	"	"	0.7	++	Good	Same	++	++	—	—	—	—	White
	Aureus.....	....	"	"	0.7	++	Meager	Better	++	++	—	—	—	—	Orange
M. S. R. Metacoccaceae	Aurantiacus.....	....	"	"	..	—	Meager	Better	++	++	—	—	—	—	Orange
	Mollis.....	....	"	"	0.7	—	Meager	Better	++	++	—	—	—	—	Orange
	Mollis.....	A	"	"	0.7	—	Meager	Better	++	++	—	—	—	—	Orange
	Citreus.....	....	Saprophytic	"	0.6	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Citreus.....	A	"	"	0.9	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Flavus.....	....	"	"	0.8	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Luteus.....	....	"	"	0.7	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Flava.....	....	"	Packets	..	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Flava.....	A	"	"	0.7	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Flava.....	B	"	"	0.7	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
R. Metacoccaceae	Citrea.....	....	"	"	..	—	Abundant	Poorer	—	—	—	—	—	—	Yellow
	Aurantiacæ.....	....	"	"	0.8	—	Abundant	Poorer	—	—	—	—	—	—	Orange
	Roseus.....	....	"	"	0.8	—	Good	Poorer	—	—	—	—	—	—	Red
	Roseus.....	A	"	"	0.8	—	Good	Poorer	—	—	—	—	—	—	Red
	Fulvus.....	....	"	Irregular groups	..	—	Abundant	.....	—	—	—	—	—	—	Red

erties resemble the sarcinae and are therefore classed with that group, in spite of their formation of orange pigment.

The yellow-pigment group is represented by 15 strains which present a very distinct and definite picture. In its abundant growth, generally gram-negative reaction, and lack of fermentative power, this group corresponds to the genus *Micrococcus*. Seven of the strains which form packets are classed with the three orange packet formers under the genus *Sarcina*.

Five strains produce a red pigment. These, in abundant growth, gram-negative reaction, reduction of nitrates, and lack of fermentative and liquefying power, form a distinct group defined by the Winslows as the genus *Rhodococcus*.

Two new biochemical tests have been applied in this study. The value of saccharose as a differentiating test among the cocci is doubtful. The ammonification of peptone promises, however, to be a valuable additional test both for generic and specific differentiation. This reaction merits further study.

The broad generic outline as laid down by the Winslows is shown to be valid and well established. Their specific types are, however, apparently too broad and inclusive and further study may bring to light new type centers. My work justifies the recognition of *Alb. ureae* and *S. aurantiaca* as distinct species and points to the probable existence of several others. More exhaustive study along this line is highly desirable.

A summary key to the genera and species of Coccaceae is given in Table 9, p. 451.